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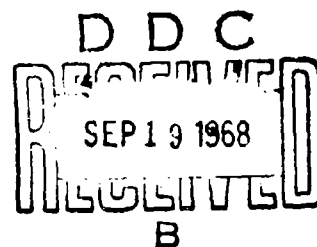
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DEPARTMENT OF THE ARMY  
Fort Detrick  
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## THE USE OF SEROLOGICAL-IMMUNOBIOLOGICAL METHODS IN THE DIAGNOSIS OF TYPHOID CARRIERSHIP

Following is a translation from Slovak of an article by J. Karolček, I. Odler, M. Draškovičová, D. Lužová, from the Institute of Epidemiology and Microbiology in Bratislava. Published in the Časopis Epidemiologie, mikrobiologie a imunologie (Journal of Epidemiology, Microbiology, Immunology), XII-4-1963, pp. 215-219./

In the present communication we are attempting to add to our previous knowledge achieved in the immuno-biological study of the typhoid carriership, in which we determined significantly increased values for the indicators of specific phagocytic activity (PA) of blood (so-called phagocytic index-PI) and significantly lower values of indicators of specific bactericidal activity of serum against *S. typhi* in vitro (so-called bactericidal index-BI) in typhoid carriers as compared to non-carriers with typhoid anamnesis and with persons without any typhoid anamnesis (Karolček et al., 1, 2).

On the basis of these results we pointed to the possibility of utilizing the determination of specific PI of the blood and specific BI of the serum in the diagnosis of typhoid carriership.

The material which follows illustrates this possibility and points to its significance. At the same time we are presenting the methodical approach to these determinations.

### Materials and Methods

Results of determinations of the specific phagocytic activity were obtained from 83 culturally proven carriers and from 126 non-carriers with typhoid anamnesis, in both cases more than one year following their recovery from illness. In addition, samples were taken from 218 persons without a proven typhoid anamnesis and not inoculated against typhoid.

Results of the determination of the specific BA were obtained from 193 carriers, 144 non-carriers with a typhoid anamnesis (same time since recovery as above) and 174 persons without a proven typhoid anamnesis and not inoculated against typhoid.

In the carriers the presence of Vi antibodies was likewise determined. The persons investigated came partly from Bratislava and other sectors of Western Slovakia, and partly from other sectors of Slovakia. All the persons were adults (18 years and up).

In determining the specific PA of the blood we use the following method:

Into a test tube of 10 mm diameter, which had been coated with a thin film of paraffin, we place 0.1 ml of the bacterial suspension in a 3.8% solution of sodium citrate. The bacterial suspension has the density of 1 billion/ml of a 24-hr. agar culture of a standard strain of *Salmonella typhi* (Ty2). The density of the suspension is determined either using standard opacities (Wellcome), or by measuring in a spectrophotometer at a wave-length of 465 (Coleman) or sometimes on another instrument. 0.1 ml of the blood being tested is added to the 0.1 of the bacterial suspension and after mixing is placed in a water bath at 37°C, where it incubates for half an hour. During this time it is thoroughly mixed by shaking every 5 minutes. After that a smear is prepared on a slide, which is then stained with Giemsa. The individual phagocytized bacteria are then counted in 50 leucocytes. This number prorated to one leucocyte gives the PI value.

Blood for this determination is collected in the morning, fasting.

In determining the specific BA of the blood we use a slight modification of the method of Pillemer et al., (5) which then appears as follows:

To a standard amount (0.4 ml) of native serum (fresh or stored at the temperatures of -40 to -70°C) we add 0.1 ml of the bacterial suspension from a 20-hour agar culture of a standard strain of *Salmonella typhi* (Ty2) in a physiological saline solution at pH 7.2 with the addition of  $MgCl_2 \cdot 6 H_2O$ , up to the concentration of 0.06%. The density of the bacterial suspension which was used equaled 200 million bacterial units per ml.

Table 1  
Values of specific PI in typhoid carriers compared with persons with a typhoid anamnesis and persons without a typhoid anamnesis

Group being investigated	Number of people	Arithmetic average and median error in average PI values
Carriers of Bacilli	83	1.25 $\pm$ 0.10
Persons with a typhoid anamnesis	126	0.12 $\pm$ 0.02
Persons without a typhoid anamnesis	218	0.06 $\pm$ 0.01

After agitating, the mixture is incubated in a water bath at 37°C for 2 hours. After that the mixture is cooled at 0°C (this serves to end the bactericidal action) and diluted serially, always fivefold. Samples of 0.1 ml

of individual dilutions are inoculated into a Petri dish of agar which had been dissolved and cooled at 45°C. After incubation for 48 hours at 37°C we count the number of colonies appearing in the smallest dilution, where it is still possible to count them well.

The degree of bactericidal activity is expressed by means of the bactericidal index (BI) which is equal to the logarithmic ratio of the initial count (P) of live organisms (control suspension without serum) to the final count (K) of live organisms (suspension with serum) after an appropriate recount of the dilution -  $BI = \log \frac{P}{K}$

In determining VI antibodies we use the hemagglutination test of Spaun (4), which had been some time ago tested and reported in our country by Matějovská (3).

#### Results

Table 1 shows average values of specific PI in typhoid carriers compared to persons with a typhoid anamnesis and without a typhoid anamnesis. The value in typhoid carriers is seen to be significantly higher than the value in the two further groups ( $p < 0.001$ ).

Table 2 shows the distribution of the PI values according to their height in individual groups of people.

We see that out of the total number of 83 carriers we find no examples with a zero value of PI, about one-quarter of persons have a lowest value of 0.5, one-quarter highest value of 0.5, but lowest of 1.0, and about half of persons investigated have an PI value higher than 1.0.

Table 2

Distribution of values of specific PI in typhoid carriers compared to persons with typhoid anamnesis and persons without a typhoid anamnesis

Group being investigated	Number of persons	Examples with PI value			
		less than 0	0.5	from 0.5-1.0	higher than 1.0
Carriers of bacilli	83	0	20	21	42
Persons with typhoid anamnesis	126	27	92	7	0
Persons without a typhoid anamnesis	218	78	136	4	0

Table 3

Values of BI of serum in relation to S.typhi in typhoid carriers compared to persons with a typhoid anamnesis and persons without a typhoid anamnesis		
Group being investigated	Number of persons	Arithmetic average and median error of average values of BI
Carriers of Bacilli	193	1.25 $\pm$ 0.08
Persons with a typhoid anamnesis	144	3.01 $\pm$ 0.06
Persons without a typhoid anamnesis	174	2.03 $\pm$ 0.07

Contrary to this, among non-carriers with a typhoid anamnesis one finds the larger proportion of cases with values of zero and less than 0.5, and only 7 cases out of 126 showed a value higher than 0.5, but lower than 1.0.

Similarly in persons without a typhoid anamnesis, where only in 4 cases out of 218 does one find values of PI higher than 0.5.

When we combine the examples of non-carriers, we see that among 344 persons cases where PI was higher than 0.5 occurred only 11 times, which represents 3.2%.

Table 3 shows average values of BI serum in typhoid carriers as compared with persons with a typhoid anamnesis and persons without a typhoid anamnesis. The BI value in carriers was determined to be significantly lower than the same value in both of further groups of persons ( $p < 0.001$ )

In Table 4 we see the distribution of the values of BI serum according to their height in individual groups of persons.

Out of 193 typhoid carriers a little more than half of the cases showed values of BI higher than 1, about a quarter of the cases values less than 1, and a little more than quarter showed zero values of BI.

Contrary to this, among 144 non-carriers with a typhoid anamnesis we found practically no examples with a BI value lower than 1 and in 174 persons without a typhoid anamnesis only a small part showed such values and there was no case recorded in which the BI value was zero.

Follow-up on titers of Vi antibodies in typhoid carriers showed that out of 84 persons investigated in 12 cases the titer was lower than 1:10, that is in about 14% (out of these in 3 cases no Vi antibodies were found), in 20 cases the titer was 1:16, in remaining cases it was higher (up to 1:128).

Table 4

Distribution of values of BI serum against S.typhi in typhoid carriers compared to persons with typhoid anamnesis and persons without a typhoid anamnesis					
Group being investigated	Number of persons	Examples with BI values			
		0	less than 0.5	from 0.5-1.0	higher than 1.0
Carriers of Bacilli	193	50	26	18	99
Persons with a typhoid anamnesis	144	0	0	0	144
Persons without a typhoid anamnesis	174	0	4	20	150

#### Discussion and Conclusions

When we compare the findings of the values of specific PI in groups of persons without carriership and those of carriers we see that in the first group PI values higher than 0.5 occur seldom and values higher than 1 are practically unknown. From this one can judge that PI values higher than 0.5, and especially values higher than 1.0, testify to the considerable probability of carriership, while taking into consideration such reservations as are necessary in valuation of the VI antibody findings. Thus one may hypothesize that an increase of a specific PI may indicate a presence in the organism (particularly in the digestive tract) of bacteria with an antigenic structure related to S.typhi; further it could mean that there had been a recent contact with a typhoid infection in the form of an abortive or inapparent infection, inoculation against typhoid, etc. In a certain number of cases where increased PI values are found it is necessary to consider the possibility of a hidden typhoid carriership.

On the other hand, one should emphasize that low values of specific PI in no way eliminate possibility of carriership.

The fact that we were able to determine the frequency of occurrence of higher values of PI in non-carriers, and low values in carriers should not in any way be considered conclusive because of the small number of investigations (especially among carriers). One must also remember the fact that in an endemic terrain the occurrence of higher values would be more frequent than in a region with a low occurrence of the infection.

What we can conclude after considering the results of investigations of specific BA sera is that the finding of BI values lower than 0.5, and especially where its values were zero, and also values lower than 1.0 in persons with a proven typhoid anamnesis, testifies to the considerable probability of carriership. At the same time, of course, higher values of BI do not mean that carriership can be eliminated.

It is obvious that here the above mentioned numerical boundaries cannot be taken mechanically and in evaluating a finding one must apply among others the above mentioned criteria.

It remains for us to note how we could facilitate the investigation of specific PI and BI in the diagnosis of individual cases, particularly in cases where we determine insufficiently definitive titers of Vi antibodies.

As we already mentioned above, we found in 12 cases out of 84 examined carriers the Vi antibody titer to be lower than 1:10 and in 20 cases the titer to be 1:16.

In the first 12 cases we were able to confirm the diagnosis of carriership with the help of determination of PI, and in 7 cases with the help of determination of BI.

We were able to confirm the diagnosis in 15 out of 20 cases with a moderately low titer of Vi antibodies.

These possibilities can be explained on the basis of a fact repeatedly determined by us, that there is no quantitative correlation between values of PI and BI on the one hand and titers of specific agglutination antibodies (not only Vi, but also O and H) on the other hand. This means that given low titers of antibodies we might find high values of PI, and either high or low values of BI.

We think that the aid for the diagnosis of carriership which we have demonstrated above does not lack in significance. At the same time we must not forget that all indirect diagnostic methods have only a relative value and that the best and safest proof of carriership still remains a finding of a positive culture.

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#### Summary

This report ties in with the previous findings of the authors, which were obtained in immunological study of typhoid carriers. In this study they found significantly higher values of indicators of specific phagocytic activity of the blood (so-called phagocytic index-PI) and significantly lower values of the indicators of bactericidal activity of the sera (BA) toward *S. typhi* in vitro (so-called bactericidal index-BI) in typhoid carriers as compared to non-carriers with a typhoid anamnesis and persons without a typhoid anamnesis.

On the basis of these findings and of further analysis of the material the authors recommend the use of determination of specific PI, as well as BI as helpful tests in the diagnosis of typhoid carriership, following the agglutination tests for Vi, as well as hemagglutination test for Vi. They particularly recommend their use in cases where the Vi agglutination titer is inconclusive.



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